Fabrication of a gel particle array in a microfluidic device for bioassays of protein and glucose in human urine samples

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This paper describes a simple method for fabricating a series of poly(ethylene glycol) diacrylate (PEG-DA) hydrogel microstructures inside microfluidic channels as probe for proteins and glucose. In order to demonstrate the feasibility of this newly developed system, bovine serum albumin (BSA) was chosen as a model protein. PEG microcolumns were used for the parallel detection of multiple components. Using tetrabromophenol blue (TBPB) and the horseradish peroxidase/glucose oxidase reaction system, bovine serum albumin (BSA) and glucose in human urine were detected by color changes. The color changes for BSA within a concentration range of 1–150 μM, and glucose within a range of 50 mM–2 M could be directly distinguished by eyes or precisely identified by optical microscope. To show the practicability of the gel particle array, protein and glucose concentrations of real human urine samples were determined, resulting in a good correlation with hospital analysis. Notably, only a 5 μL sample was needed for a parallel measurement of both analytes. Conveniently, no special readout equipment or power source was required during the diagnosis process, which is promising for an application in rapid point-of-care diagnosis. © *2011 American Institute of Physics*. [doi:10.1063/1.3623412]

I. INTRODUCTION

Modern development of health care systems requires new technology in the challenging field of point-of-care diagnosis. Recently, efforts were made for developing a portable and rapid diagnosis method. The keys to control the spread of diseases and apply the right medical treatment are rapid and accurate diagnosis tools, which are permanently improving in the economically developed world. However, there are still several difficulties preventing a wide application of modern techniques in developing countries.[1,2] To solve these problems, researchers started using inexpensive and facile materials to perform bioassays.[3–7] Rapid detection approaches were developed, such as capillary driven flow[8] and chemical signal amplification.[9] In recent years, Whitesides *et al*. used an egg beater as a centrifuge[10] and invented paper-based microfluidic devices,[11–13] which provided a new concept for developing a portable and inexpensive diagnosis device. Microfluidic devices using paper substrate greatly reduced time and reagent consumption. However, the approach of photolithography on paper substrate by using SU-8 photoresist is complex and time consuming, and each paper indicator could be used only once. Thus, we try to develop an alternative way by fabricating a PEG hydrogel microstructured array inside microfluidic devices, which can be reused.

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The quantification of proteins, peptides, nucleic acids, and carbohydrates within very low sample volumes in many cases is a difficult challenge, which requires high throughput and rapid, efficient screening.[14–17] In recent years, two methods have received the most attention use and development: planar arrays[18–21] and suspension arrays.[22–24] Description diabetes mellitus is for a whole group of diseases, which in addition to a high blood sugar causes the excretion of sugar in urine. The glucose concentration in urine can be an easily indication on diabetes. The content of protein in urine can also be considered as an indicator of diabetes, because the kidney function is harmed by diabetes. Considering the clinical requirement for rapid diagnosis of diabetes, glucose and proteins were selected as the two components to be measured in this work. Several approaches were developed for rapid detection of glucose, such as gold nanoparticles,[25,26] quantum dots,[27,28] and enzyme catalytic activity.[29,30] For the protein detection assay, alternative methods were developed to obtain a sensitive measurement.[31,32]

Traditional methods for human urine detection often need a lot of samples and a pretreatment before the detection. However, these conventional methods require a large number of human urine, reagents, and complication sample pretreatment. The micrototal analysis systems have been a growing interest for analytical chemistry since microfluidic technology provides several advantages such as low reagent consumption, shortening analysis time, and miniaturization. And the motivation and usability of the system can be described as follows: a device combined with microchips is committed to realizing rapid detection with low consumption, high sensitivity, and accurate definition, while the PEG microcolumns modified to the microfluidic chip and the chip itself spend little and are fabricated easily; thus, it is potential to be applied in analysis of environmental and biological samples.[33–35]

In our previous work, we developed a PEG hydrogel microcolumn array technique to provide a convenient and suitable platform to perform biochemical assays.[36] PEG is a convenient material for the fabrication of microarrays, because of its non-volatile, recyclable and inert character.[37] Additionally, PEG has a low toxicity and is biocompatible, and thus a large number of applications on biochemical assays could be carried out after the cross-linking induced by ultraviolet light.[38,39] Modified PEG particles containing sensing reagent capsules can be used as a probe, which avoids time consuming surface modifications during the fabrication process. For example, hydrophilic PEG-DA gel particles functionalized with *p*H indicator was used to measure the *p*H value of the environment of living cells.[40] This development demonstrated the capability of PEG-DA gel particles for high throughput analysis with a small sample volume and its high potential for the application in *in-situ* analysis.

Here, we developed a rapid and simple method to fabricate a PEG-DA hydrogel microcolumn array for bioassays. Considering a simple readout, tetrabromophenol blue (TBPB) and the horseradish peroxidase/glucose oxidase system were chosen for the detection of proteins and glucose, respectively, via the color changes. This method provides a facile, low-cost, and portable way to fabricate probed particles and perform chemical assays. A rapid determination of protein and glucose concentration in human urine was realized using only microliter volumes of sample. These, PEG microcolumns provided a high potential for rapid diagnosis in developing countries by meeting point-of-care requirements, while also being simple and cheap to produce.

II. EXPERIMENTAL

A. Reagents and materials

Poly(ethylene glycol) diacrylate (PEG-DA, molecular weight of 700 Da) and 2-hydroxy-2-methylpropiophenone (HMPP) photoinitiator were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). The PEG precursor solution with 1.5% photoinitiator was stored at 4 °C before mixing with the other reagents. 3-(Trichlorosilyl) propyl methacrylate (TPM) was obtained from Fluka Chemicals (Milwaukee, WI, USA). Ethanol (HPLC grade) was purchased from Fisher (New Jersey, NJ, USA). The tetrabromophenol blue (TBPB) diluted in 95% ethanol to 3.3 mM was obtained from Sigma-Aldrich. Citrate buffer solution (*p*H 1.8) was prepared by dissolving 0.25 M sodium citrate in deionized water. Horseradish peroxidase and glucose

oxidase were obtained from Beijing Boisynthesis Biotechnology Co., Ltd. (Beijing, China). The protein used in all assays was bovine serum albumin (BSA) produced by Kehaoda (Beijing, China). Glucose was purchased from Beijing Dingguo Co., Ltd. (Beijing, China). Silicon wafers were obtained from Xilika Crystal Polishing Material Co., Ltd. (Tianjin, China). Negative photoresist (SU-8 2050) and the developer were purchased from Microchem Corp. (Newton, MA, USA). Poly(dimethylsiloxane) (PDMS) and the curing agent were obtained from Dow Corning (Sylgard 184, Midland, MI, USA). A plasma cleaner (PDC-32G) from Harrick Plasma (Ithaca, NY, USA) was used for oxygen plasma treatment. A fluorescence microscope (Leica DMI 4000 B, Wetzlar, Germany) equipped with a CCD camera was used to induce the photopolymerization of the PEG and to obtain images of the microfluidic devices. The dimensions of each microstructure were manually measured by using Leica Application Suite, LAS V2.7.

B. Fabrication of PEG microstructures

As shown in Figs. 1(b)–1(d), a simple PEG microcolumn array was generated inside the microchannels via UV-initiated polymerization. The principle of polymerization was shown in Fig. 2(a). A fluorescence microscope equipped with an external fluorescence light source with a metal halide bulb (Leica EL6000) was used to photopolymerize the PEG precursor inside the microchannels. The UV light beam (340–380 nm) from the external fluorescence light source was focused through the objectives and projected onto the precursor inside the microchannels. An exposure with UV light for photopolymerization was realized using a computer controlled shutter on the microscope. Field diaphragms and objectives with different magnification were used to control the illumination area inside the microchannels. The size of the hydrogel

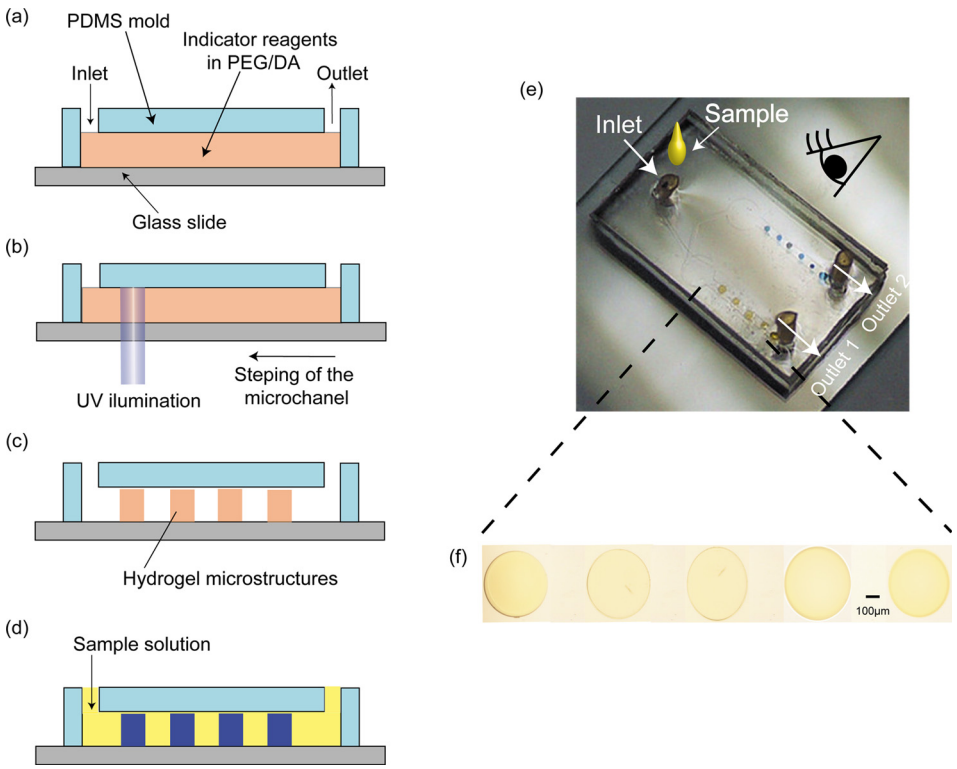

FIG. 1. Schematic illustration of the preparation of PEG hydrogel microcolumns and photopolymerization process. (a) The prepared PEG precursor containing the indicator reagents was injected into the microchannels. (b) PEG hydrogel microcolumns array was formed by *in situ* UV photopolymerization using a fluorescence microscope. (c) The unpolymerized hydrogel precursor solution was removed by buffer. (d) Urine samples were injected into the channels to obtain the color changes. (e) A photographic image of the whole microfluidic device. A Y-shape channel was fabricated for multiple components simultaneous detection. (f) An enlarged microscopic image for the detection area.

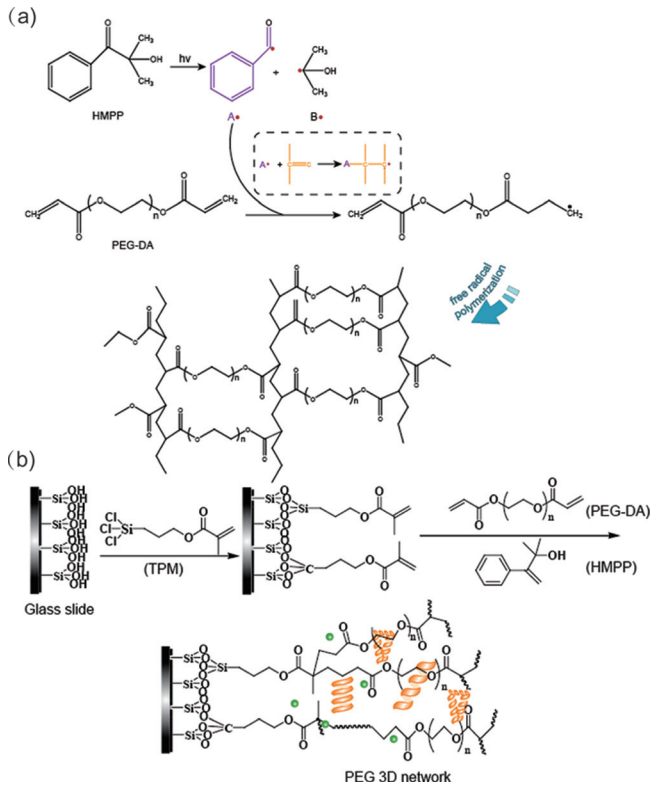

FIG. 2. Principles of UV-initiated polymerization of the PEG microcolumn array (a) and the modification of the glass surface by TPM and linked with a three-dimensional PEG hydrogel structure (b).

microstructures was controlled by objectives with different magnifications and the time of UV illumination. The distance of the adjacent edges of the hydrogel microstructures could be controlled manually or by a stepper motor.

To enhance the adhesion between the PEG hydrogel microcolumns and the glass slides, the inner glass surfaces of the microchannels were modified with a monolayer of TPM. This step was achieved by injecting a 1% (v/v) TPM solution in paraffin oil into the microchannels for 10 min followed by a rinse flow with ethanol. The microchip was placed into an oven at 95 °C for 30 min and dried with N_2 afterwards. Subsequent photopolymerization covalently linked the gel to the glass slide via the silane-coupling agent. As shown in Fig. 2(b), the PEG hydrogel was cross-linked on the TPM modified glass slide with a three-dimensional structure, which can capture some protein or small molecule by the small network capsule or adsorptive action. Briefly, the process of measuring could be described as follows: the mixture of indicator reagents and PEG-DA was injected into the prepared PDMS microchannels (Fig. 1(a)). After the program controlled exposure by UV light, the PEG hydrogel microcolumn arrays were fabricated (Fig. 1(b)). Extra unpolymerized reagents were flushed away by phosphate buffered saline (PBS) solution (Fig. 1(c)). Then, the samples were introduced into the channel, and the color changes of the PEG microcolumns were observed and recorded (Fig. 1(d)).

C. Microfluidic devices fabrication

The microfluidic devices were fabricated from PDMS produced by standard soft lithography techniques as reported.[41] Briefly, the mold of microstructures were fabricated by coating the negative photoresist SU-8 2050 at 3000 rpm spin speed (50 μm thick film) on a silicon wafer cleaned by piranha solution. After photolithographic patterning of a photoresist coated silicon wafer, a mold that carried a relief of the desired microstructure was generated. A 10:1 premixed PDMS prepolymer was prepared according to the manufacturer instructions, degassed in

a vacuum chamber for 1 h, poured on the prepared mold, and then cured in an oven at 70 °C for 2 h. The PDMS was cut from the mold with a surgical scalpel and then carefully peeled off. The channels, inlets and outlets, were punched by a flat-tip syringe needle. The channels were sealed onto a glass slide after an oxygen plasma treatment for 90 s. Or the cleaned PDMS devices were attached on to a glass slide to form a reversible sealing for a reusable purpose. The microchannels obtained with a Y-shape for the rapid detection of protein and glucose is shown in Fig. 1(e). All branch channels have a depth of 50 μm. In order to reduce the flow shearing force on the PEG microcolumns when the samples were injected into the channel, two round chambers were designed between the two branch channels and the junction of the Y-shaped channel was connected with the outlet.

D. Assays of protein and glucose

The protein assay was based on the dye-binding method. A color shift in the dye could be observed after binding to proteins due to differences in the molecular structure between binding and unbind form. The dye solution was prepared by dissolving TBPB in 95% ethanol with a concentration of 3.3 mM. The PEG-DA prepolymer was well mixed with the TBPB solution in a ratio of 3:2 (v/v) and injected into one of the branch channels from outlet 1 (Fig. 1(e)). Unpolymerized hydrogel precursor solution was removed by 250 mM citrate buffer solution (*p*H 1.8) introduced into the channels. The prepared branch channels were allowed to air dry at room temperature for further detection.

For the glucose assay, a 1/5 dilution of horseradish peroxidase/glucose oxidase solution was prepared in sterilized water to 150 units of protein per mL of solution. The PEG hydrogel precursor solution consisting of 1.5% (v/v) HMPP was mixed with the horseradish peroxidase/glucose oxidase solution and a 6 M solution of potassium iodide by the ratio of 1:3:2 (v/v). The mixed PEG hydrogel precursor and detector reagents were injected into the other branch channels from outlet 2. After the photopolymerization, the unpolymerized hydrogel solution was flushed by deionized water.

E. Reusable microfluidic devices

In order to reduce the cost of a single time rapid diagnosis, the microfluidic devices fabricated to generate the PEG hydrogel microcolumns were reused several times. During the process of photopolymerization, atmospheric oxygen diffused through the porous PDMS from the top of the whole device. Under the UV light exposure, a thin un-cross-linked film of oligomer was caused by the oxygen-induced inhibition of free radical polymerization reactions and is facilitated by the permeability of PDMS to atmospheric oxygen (shown in Fig. 3). After the detection of one batch of samples, the PDMS device can be peeled off the glass slide, cleaned, and sealed revisable on a new glass slide for a repeated use. As an alternative approach, the PDMS device could also be peeled off after generating the PEG microcolumns, since the PEG microcolumns are allowed to be dried in the air and stored in room temperature for the diagnosis. The microcolumns could be immersed in a sample to induce a color change. But in this experiment, we chose to induce the samples from the inlet of the microfluidic chip, in order to realize the detection of multiple components at the same time.

III. RESULTS AND DISCUSSION

A. PEG hydrogel microcolumns

A photo mask was required in the traditional methods to control the size of the photopolymerized hydrogel microstructures. In our experiment, a field diaphragm offered by a fluorescence microscope was used to control the UV illumination region, by which the morphology of the hydrogel microstructures, such as cylindrical and rectangular shapes, can be easily regulated. The size of the microstructures was controlled by the magnification of the objective and the field diaphragm of the fluorescence microscope.

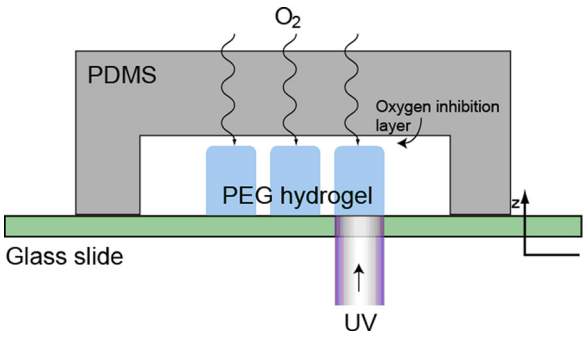

FIG. 3. Cross-sectional view of the PDMS device showing PEG hydrogel microcolumns formed using flow lithography. The UV light regulated by the field diaphragams is projected from the bottom to form microcolumns in a film of oligomer that is enclosed in the PDMS device. The microcolumns formed are adherent to the glass slide, and separated from the top walls of the device by a thin oxygen inhibition zone consisting of un-cross-linked oligomer.

At first, the relationship between the exposure time and the size of the PEG microcolumns was evaluated. As shown in Fig. 4, the objectives with magnification of 20× and 40× were used for photopolymerization in our experiments, and the 5th and 6th large field diaphragms were selected for this evaluation. Considering the size of the microfluidic channel and convenience for optical observation, a 350 ms exposure with a magnification of 20× with the 5th large field diaphragm was chosen to generate the PEG hydrogel microcolumns with a final diameter of 500 μm.

In our experiment, we injected the prepared PEG precursor mixture into the microchannels to generate the hydrogel microcolumns. Only 1.5 μL mixed reagent was required for each detection process, which contained 0.45 μL TBPB or 0.38 μL horseradish peroxidase/glucose oxidase.

B. Protein and glucose measurement

We measured artificial samples of glucose and protein in the range of 0–2.0 M for glucose and 0–400 μM for bovine serum albumin (BSA). All standard samples of glucose and BSA solutions were prepared in deionized water. The detection was achieved by simply injecting the

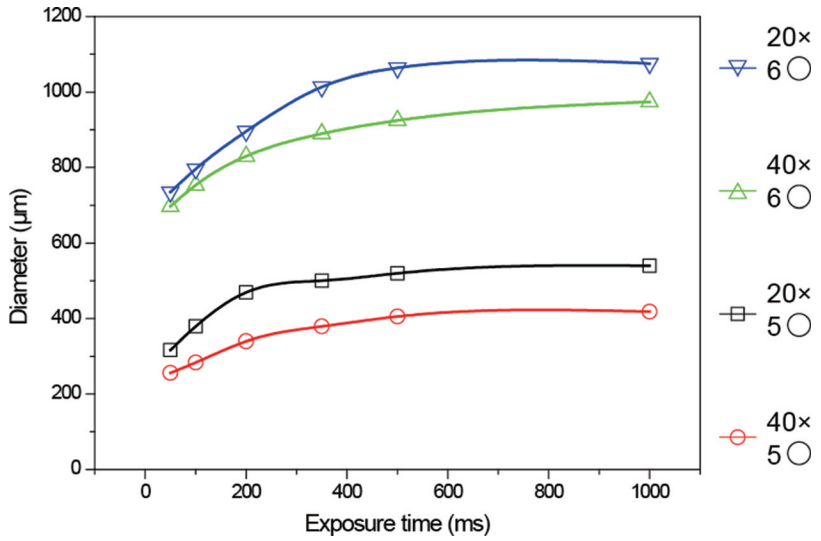

FIG. 4. The diameters of circular hydrogel microstructures generated using different configurations of photolithography. The error bars are too small to label on the plots. 10×, 20×, and 40× represent the objectives we used. 6○ and 5○ represent the field diaphragms we used.

sample into the inlet. Both of the branch channels were automatically filled by the capillary effect. The fluid filled the entire device within approximately 30 s, and the color was developed in 5 min at room temperature. In all experiments, as shown in Fig. 5, the observed color changes were directly proportional to the amount of glucose and BSA in the samples. The ordinate of the calibration curve represents the integral of light areas of each PEG hydrogel microcolumn. The intensity of each microcolumn was obtained by analysis a certain color on the image (red for glucose and blue for protein). Light intensities of each spot in the image were integrated, and the mean intensity was calculated using image-Pro Plus program. The relative standard deviation (RSD) for three parallel measurements of glucose was less than 5.1%, and the RSD of BSA was less than 4.5%.

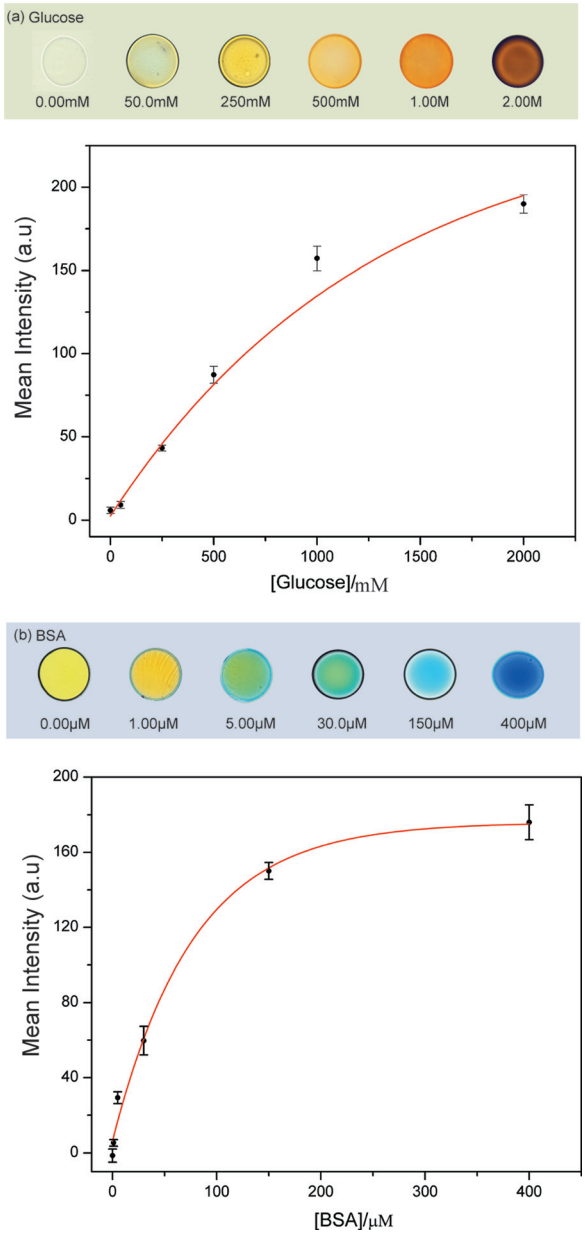

FIG. 5. Color changes evaluation obtained by varying the concentrations of BSA (a) and glucose (b). The value for each concentration was calculated by the average from five times detection, separate.

TABLE I. Comparison of the results by proposed method and hospital analysis.

Sample number	Glucose detection results	Glucose hospital results	BSA detection results	BSA hospital results
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	+	—	—
5	+	+	—	—
6	+	+	—	—
7	+	+	—	—
8	+	+	+	—*
9	—	—	+	—*
10	+	+	+	—*

+Positive; —: Negative.
*10 μM BSA solution was added into the original urine samples.

The detections of BSA and glucose could be achieved in two different procedures: samples could be flushed through the microfluidic channel using a syringe or simply dropped on the PEG hydrogel microcolumns. For low volume samples, injection through the microfluidic channel only required less than 5 μL for the parallel detection of BSA and glucose. However, this procedure will take 3 min for the entire color development. For large volume samples, the direct immerse of the PEG hydrogel microcolumns after peeling off the PDMS device provide more obvious and faster results.

C. Urine samples detection

10 urine samples were collected at the Hospital of Peking Union Medical College, containing samples from diabetes patients and healthy people. Samples were injected into the microchannels without any pretreatment. The images were taken 5 min after the sample injection, and color intensities were calculated in the same way as for the standard samples. In order to

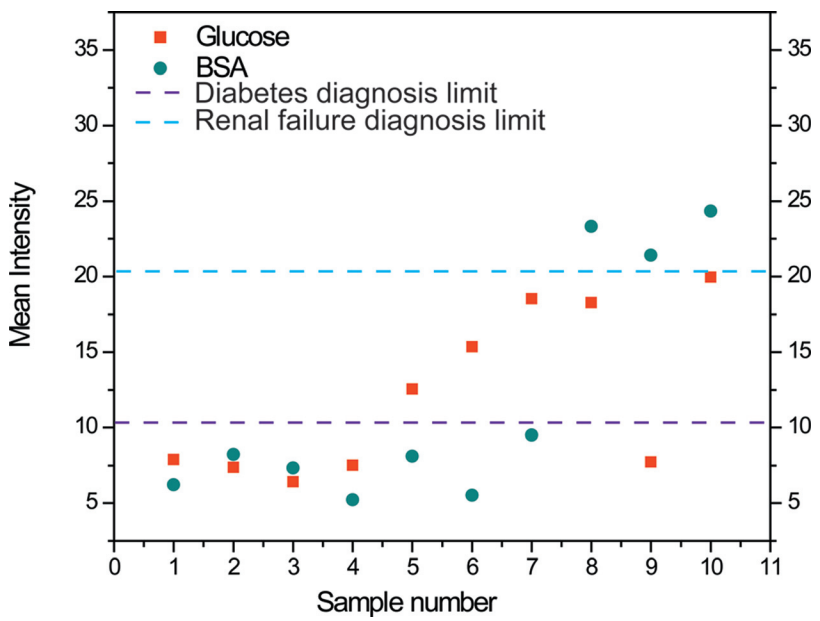

FIG. 6. Results for the quantification of proteins and glucose in real human urine samples. The dashed line indicates the clinical relevant concentration limit for diabetes diagnosis.

evaluate the protein assay, 10 μM BSA standard solution was added into the samples number 8, 9, and 10 as a positive control (see Table I). According to the clinical data, urine glucose concentrations higher than 8.8 mM indicate the potential of diabetes. Urine protein concentrations higher than 1.5 μM reveal a damage of the kidney function. As shown in Fig. 6, among all 10 samples, 5 diabetes patients were found. In comparison to the diagnosis of the hospitals physicians, 90% accuracy was achieved with the new gel particle array. A detailed comparison of the results for the real human serum samples is shown in Table I. In the case of the protein assay, all three positive control samples all showed accurate results.

IV. CONCLUSION

In summary, the PEG hydrogel microcolumn method is suitable for parallel multiple components detection simply via varying the reagents inside PEG-DA hydrogel structures. Up to ten components could be detected in parallel by designing more branch microchannels in low sample volumes in future. Furthermore, PEG hydrogel microcolumns mixed with reagents could be kept stable after being dried for several days. In addition, the measurement results could be easily distinguished optically instead of via large equipment, and thus, this technology may be promising in rural areas, which often lack electricity and skilled workers. Compared to chips based on paper substrate, our method avoided the loss of target analytes by adsorption on the paper substrate before arriving at the detection area. PEG hydrogel microcolumns can enrich the analytes due to the three-dimensional network structure. Such a low-cost, simple, and portable diagnostic platform will be helpful in those less-industrialized regions and particular situations where fast and simple tests are important for disease diagnosis and monitoring.

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